

Effect of shearing on formation of silk fibers from regenerated *Bombyx mori* silk fibroin aqueous solution

Fang Xie, Huihui Zhang, Huili Shao*, Xuechao Hu

State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Material Science and Engineering,
Donghua University, Shanghai 200051, PR China

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Abstract

In this paper, the spinnable regenerated silk fibroin aqueous solution with high concentration was prepared and the regenerated silk fibers were obtained from the aqueous solution by two different spinning processes at ambient temperature. The orientation of these fibers was characterized by polarizing microscope. Their secondary structure was investigated by Raman spectroscopy and related mechanical properties were also measured. These data showed that shearing is an important step for increasing orientation and silk II (β -sheet) structure, and the mechanical properties of the regenerated silk fibers can also be improved by shearing.

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1. Introduction

Natural silks from spiders and silkworms have attracted much attention for a long time because they exhibit high strength and toughness [1–3]. Researchers all over the world are interested in biomimicking of the spinning process of natural silk fiber and hope to produce artificial silk in vitro for industrial production [4–7]. A number of groups have attempted to spin silk fibers from regenerated silk fibroin (RSF) solution or recombinant spidroin solution. In these works, some organic solvents were used to form the dope, such as hexafluoro-2-propanol and formic acid, etc., and methanol or acetone was used as coagulation bath [7–13]. In contrast to hazardous solvents and harsh conditions needed to produce aramid fibers, in natural, however, spider silk and silkworm silk are spun into air at ambient temperature and using water as the solvent. Yamaura et al. [14] have reported the silk can be produced by forcing RSF aqueous solution through a glass nozzle, but the mechanical properties of the obtained fibers were not reported and the fibers lacked the silk II structure of natural silk. Shao et al. [15] showed that the regenerated spider filament could be drawn out by a needle from the

surface of a 0.08% aqueous solution of regenerated spidroin. Its tensile strength was 0.11–0.14 GPa and the initial modulus was around 6.0 GPa, while breaking elongation varied between 10 and 27%.

The silk gland of silkworm consists of three relatively distinct regions. The concentrations of silk fibroin in the posterior, middle and anterior portion of the gland are 15, 25 and 30 wt.%, respectively. In addition, the pH value of the solution and the content of calcium ion are also varied in these three portions. To mimic the spinning process of silkworm, it would be desirable to prepare a solution, in which the concentration, pH value and ions are similar to those of silkworm, and then to spin the solution directly into air.

Generally speaking, silk fibroin has three conformations, i.e. random coil, α -form (silk I) and β -sheet (silk II) structure [6]. Yamaura et al. [14], Iizuka [16] and Vollrath and Knight [2] showed the spinning process of silkworm involves applying shear and elongational stress on the silk fibroin aqueous solution in the gland, causing silk fibroin in aqueous solution to crystallize and transfer conformation from random coil or silk I to silk II. However, few studies have been made on the effect of shearing on regenerated silk fibers from concentrated RSF aqueous solution.

As a prime work, the spinnable RSF aqueous solution with high concentration but without adjusting pH value and ions

* Corresponding author. Tel.: +86 21 62373512; fax: +86 21 62378944.
E-mail address: hlshao@dhu.edu.cn (H. Shao).

content was prepared and the regenerated silk fibers were obtained from the concentrated aqueous solution by two different spinning processes. The mechanical properties were measured and Raman analysis was applied to study the effect of shearing on regenerated silk fibers.

2. Experimental

2.1. Preparation of concentrated RSF aqueous solution

To remove the sericin, the cocoons of *Bombyx mori* were degummed twice with boiling aqueous 0.5 wt.% Na_2CO_3 solution for 30 min each, and then washed thoroughly with deionized water and dried at room temperature to prepare degummed natural silk fibers. Subsequently the degummed silk fibers were dissolved in a 9.3 M LiBr aqueous solution at ambient temperature to a concentration of 10 wt.%. After being diluted with four times of water, the diluted fibroin solution was dialyzed against deionized water for 3 days with a cellulose semi-permeable membrane (molecular weight cut off (MWCO): $14,000 \pm 2000$) to remove the salt. Finally, the concentrated RSF solution (ca. 39 wt.%) was obtained by gentle evaporation of water from the dilute RSF aqueous solution in constant low temperature, which ensured without shear and no solid precipitation.

2.2. Samples preparation

In order to investigate the effect of shearing, the 39 wt.% RSF aqueous solution was injected into the capillary (diameter $530 \mu\text{m}$) (shear rate is around 28 s^{-1}) and solidified at ambient temperature. The shear rate was calculated by Hagen-Poiseuille equation [17]. The solidified RSF solution in the capillary was denoted as sample A.

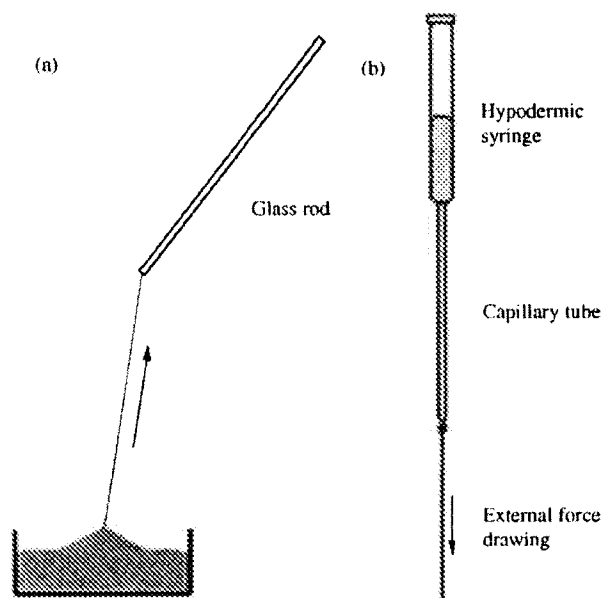


Fig. 1. Schematic devices for preparation of regenerated silk fibers: (a) sample B; (b) sample C.

The devices illustrated in Fig. 1 were used to obtain the regenerated silk samples at 25°C and R.H. 40%. As shown in Fig. 1(a), to prepare sample B, the prepared concentrated RSF aqueous solution with a concentration of 39 wt.% was placed in a proper vessel, and a glass rod with a diameter of 3 mm was immersed in the aqueous solution to a depth of 4 mm from the top surface. Subsequently, the rod was raised to draw a filament at a speed of around 1 cm/s , until it was broken and the filament was immediately dried in air, the regenerated silk fiber was formed. The filaments of sample B are uneven. Fig. 1(b) shows the way to prepare sample C. The above RSF aqueous solution was injected into a hypodermic syringe. A capillary with an inner diameter of $530 \mu\text{m}$ and a length of 20 cm was directly connected with the hypodermic syringe. Then the RSF aqueous solution in hypodermic syringe was pushed into the capillary (shear rate is around 65 s^{-1}). A droplet was formed always at the outlet of the capillary when the RSF aqueous solution was extruded from “spinneret” of the capillary. Then the droplet was hold and a continuous silk was drawn into the air from the solution at a speed of around 1 cm/s at ambient temperature, sample C was obtained. Compared with sample B, sample C is more even.

2.3. Optical and birefringence measurements

The appearance and diameters of these fibers were determined using an Olympus BX-51 polarizing microscope (Japan). The birefringence of the samples was measured under the polarizing light with the aid of a Model CTB Berek compensator (Japan).

2.4. Raman spectroscopy

The Raman spectra were obtained using a Dilor LabRam-1B Raman microscope (France) from single filaments of regenerated silk or RSF aqueous solution that had been fixed on the appropriate viewing frames. A He-Ne laser was used to give 6 mW of energy at 632.8 nm red line. Spectra were recorded from 900 to 1800 cm^{-1} with a 2 cm^{-1} spectral width resolution.

2.5. Mechanical properties

The mechanical properties of two regenerated silk fibers (samples B and C) and natural silk were tested on an Instron 5565 tensile testing instrument (USA). The filament with 20 mm gauge length was fixed between the two hooks of the instrument and then was stretched at a rate of 2 mm/min . The measurements were carried out at 20°C and R.H. 65%, and each value represents an average of 10 measurements. The measurement of tensile properties followed the ASTM D 3822 method.

3. Results and discussion

3.1. Polarization and orientation of regenerated silks

When silkworm is spinning, the silk fibroin aqueous solution is subjected to both shearing and drawing. Shearing force is applied on the silk fibroin as it flows through the gland. While the

drawing occurs after the silk fibroin is exited from the spinneret [2,16]. Here, the intent of preparation of sample C is to mimic the spinning process of silkworm, because the shearing force was applied by pressing the RSF aqueous solution through a capillary, like the gland of silkworm. And then the RSF aqueous solution was drawn by external force. As a comparison, sample A was made just by shearing without drawing, and sample B was made just by applying drawing and without shearing.

Fig. 2 shows the polarizing micrographs of regenerated silk fibers (samples B and C). The polarized light of sample B (without shearing) can be observed, but its degree of orientation was too weak to be detected. While it was found that the solidified RSF aqueous solution (sample A) flow through a capillary also showed weak birefringence although only shearing was applied (shear rate is around 28 s^{-1}). Its optical birefringence value reached 0.0067, which is attributed to the orientation

of fibroin along the capillary. In addition, sample C showed enhanced birefringence because of the combination of shearing (shear rate is around 65 s^{-1}) and drawing, the value was measured as $\Delta n = 0.009$, and higher than that of sample A. Thus, the orientation degree of these samples is in the following order: sample C > sample A > sample B. This phenomenon confirmed that the shearing is important for the orientation of regenerated silk fibers.

Since the birefringence could not be detected in sample B, it can be speculated that without shearing the conformational transition from random coil/silk I to silk II structure of silk fibroin in the solution was rather weak that leads to a low orientation of sample B, in spite of the sample has been subjected to drawing. As to sample C, firstly it has experienced the shearing that was induced by pressing the RSF aqueous solution through the capillary and the shearing make more random coil/silk I conformation transform to silk II structure, then it has been drawn by external force. This process increased the degree of orientation.

3.2. Secondary structure of regenerated silks

Raman spectroscopy is a powerful tool to investigate the secondary structure of proteins and has already given important insights into the structure of natural silk filaments of silkworms [18]. All the secondary structures of proteins (silk I, silk II, and random coil structures) have characteristic bands at amide I ($1680\text{--}1640\text{ cm}^{-1}$) and amide III ($1270\text{--}1220\text{ cm}^{-1}$) [18–20].

Fig. 3 shows a comparison between the Raman spectra of degummed natural silk and regenerated silk fibers. In amide III region, two regenerated silk fiber samples presented distinct characteristic band of random coil (1249 cm^{-1}) and weak silk I band (1268 cm^{-1}). At the same time, samples B and C exhibited characteristic band of silk II structure (1232 cm^{-1}) in this region and the intensity of sample C is stronger than that of sample B. In amide I region, it can be found that both regenerated silk fibers mainly presented characteristic band of silk II structure

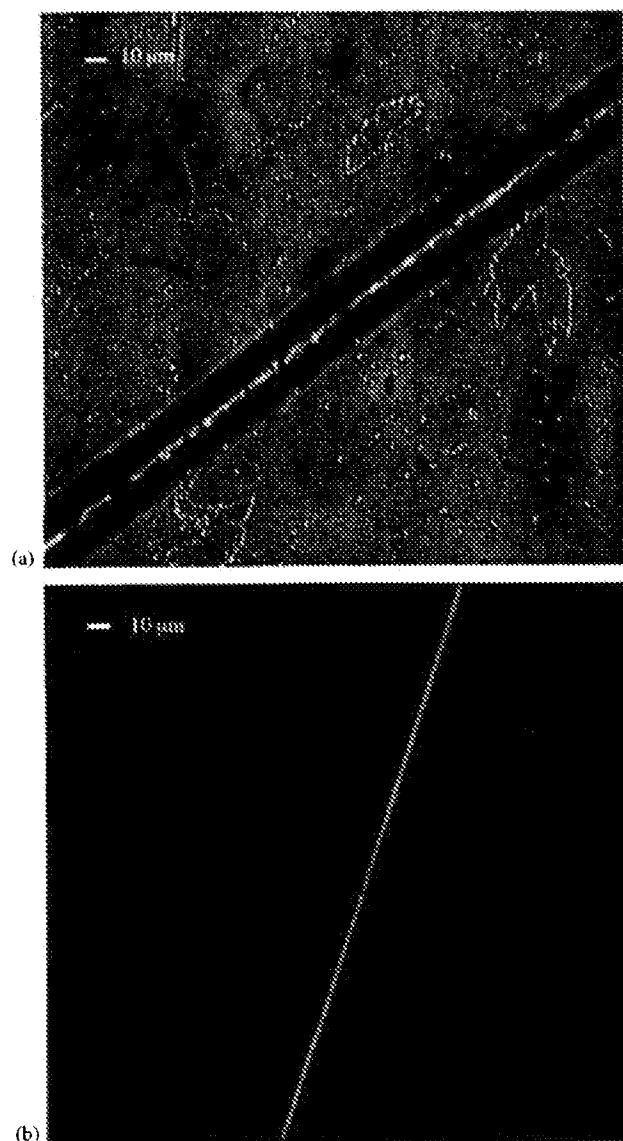


Fig. 2. Polarizing micrographs of regenerated silk fibers from aqueous solution: (a) sample B; (b) sample C.

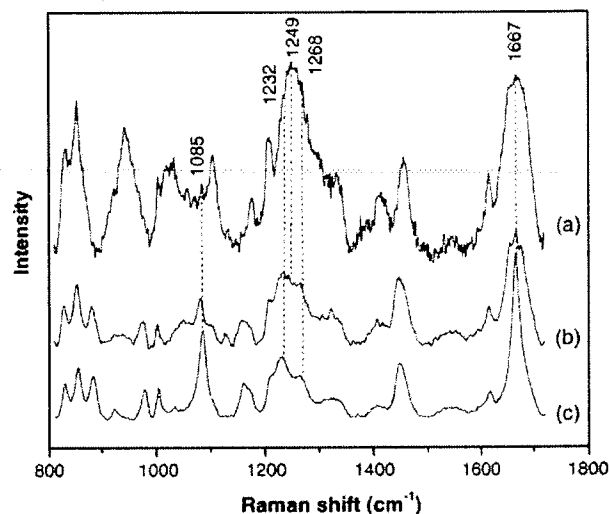


Fig. 3. Raman spectra of regenerated silk fibers: (a) sample B; (b) sample C; (c) degummed natural silk.

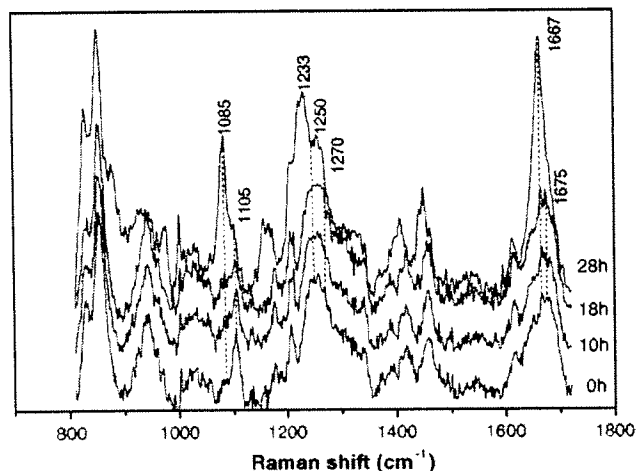


Fig. 4. Raman spectra of RSF aqueous solution with different flowing time.

(1667 cm^{-1}) and the band is sharper for sample C than sample B. In addition to that, samples B and C had a sensitive band at about 1085 cm^{-1} , which also indicated that silk II conformation existed again. On the other side, the degummed natural silk (Fig. 3c) exhibited three major characteristic bands of silk II at 1085, 1232 and 1667 cm^{-1} , indicating that silk fibroin assumed complete silk II structure. This result is also in agreement with the results of the FT-IR, NMR and X-ray methods by others [9–11,21,22]. By comparing these three samples, it is obvious that there is no complete silk II structure in regenerated silk fibers as that of the natural silk. It indicated that these regenerated silk fibers have a mixture conformation of random coil/silk I and silk II.

When samples B and C was compared, it can be found that sample B had stronger characteristic bands of random coil (1249 cm^{-1}), whereas sample C had stronger characteristic bands of silk II structure (1232, 1667 and 1085 cm^{-1}) than sample B. It showed that sheared sample C have more silk II structure than unsheared sample B.

3.3. Conformation of silk fibroin in sheared concentrated RSF aqueous solution

Yamaura et al. [14] and Iizuka [16] have presented evidence that shear influences greatly the conformational transition of silk fibroin during the spinning process of silkworm. In order to further confirm the conformational feature of RSF in sheared aqueous solution, the freshly prepared RSF aqueous solution with a concentration of 33 wt.% was pressed to the above capillary and kept it flow. The Raman spectra of the RSF aqueous solution with different flowing time were shown in Fig. 4.

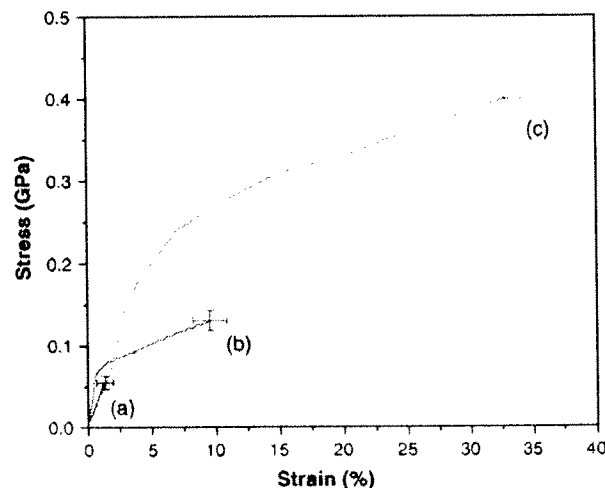


Fig. 5. Stress-strain curves of regenerated silk fibers: (a) sample B; (b) sample C; (c) natural silk.

When the flowing time was less than 18 h, the band at 1250 cm^{-1} (random coil) was reduced in intensity with the increasing of flowing time. At the same time, the characteristic bands of silk I at 1105 and 1270 cm^{-1} also became weaker. However, the bands at 1085, 1667 and 1675 cm^{-1} (silk II) were gradually enhanced with the increasing of flowing time. After flowing 28 h, the Raman spectra exhibited two major characteristic bands of silk II at 1233 and 1667 cm^{-1} , indicating that silk fibroin were mainly in silk II structure. It was concluded that the concentrated RSF aqueous solution in the capillary undergoes a conformational change from random coil/silk I to silk II structure owing to mechanical shearing.

It was speculated that the silk fibroin chains were forced to extend under the shearing during flowing [23] and the molecules would then be easier to align with the flowing direction. Due to the sheared solution has more order, which makes the sample C has more silk II structure and better mechanical properties. That is to say, shearing is an important step for the formation of a filament.

3.4. Mechanical properties of regenerated silks

The stress-strain curves of the regenerated silk fiber samples were shown in Fig. 5. Their mechanical properties were summarized in Table 1.

From Fig. 5 and Table 1, it can be found that sample C showed much better mechanical properties than sample B. And the tensile strength of sample C was about 0.13 GPa, which was close to regenerated spider silk (0.11–0.14 GPa) [15]. It is well known

Table 1
Mechanical properties of regenerated silk fibers

Sample	Breaking elongation (%)	Tensile strength (GPa)	Initial modulus (GPa)	Birefringence (Δn)
Sample B	1.22	0.05	3.54	–
Sample C	9.55	0.13	7.18	0.009
<i>Bombyx mori</i> silk	15–35	0.2–0.6	5–12	0.057

that the mechanical properties of the fiber are related to its degree of orientation. As analyzed previously, due to the combination of shearing and drawing, sample C had comparatively higher degree of orientation that resulted in much better mechanical properties. The poor mechanical properties of sample B also indicated that the well-orientated silk with good mechanical properties cannot be formed only with drawing.

Indeed, neither the tensile strength nor the breaking elongation of the regenerated silk could compare with those of natural silk when such simple process was used. The poor mechanical properties of the regenerated silk fibers can be attributed to the different spinning processes. The regenerated filament was prepared by simply shearing and drawing from isotropic silk fibroin aqueous solution without adjusting pH value or adding any ions; while the natural silk was produced using several sophisticated spinning techniques from anisotropic liquid crystalline solution [16].

Hence, in order to prepare a high performance RSF filament, the next steps should be (1) to mimic the silkworm to prepare a RSF dope that has similar anisotropic properties as the native silk fibroin solution; (2) to improve spinning techniques.

4. Conclusions

The regenerated silk fibers can be obtained from concentrated RSF aqueous solution without any organic solvents being used and the fiber is spun into air at ambient temperature just like the spider or silkworm done. The birefringence of the samples showed that the degree of orientation could be increased by shearing. From Raman spectra analysis and mechanical properties measurement, it also can be concluded that unlike the spidroin aqueous solution [15], the shearing is important for increasing silk II structure and improving mechanical properties of the regenerated silk fibers.

However, such regenerated silks obtained from the aqueous solution without adjusting pH value, ionic type and concentration displayed poor mechanical properties than that of natural silkworm silk. To improve the mechanical properties of regenerated silk fibers, the techniques of mimicking the spinning process should be further developed and it is very important to obtain the dope that has similar anisotropic properties as the native silk fibroin solution.

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References

- [1] E. Atkins, *Nature* 424 (2003) 1010.
- [2] F. Vollrath, D.P. Knight, *Nature* 410 (2001) 541–548.
- [3] Z.Z. Shao, F. Vollrath, *Nature* 418 (2002) 741.
- [4] D.A. Tirrell, *Science* 271 (1996) 39.
- [5] A. Lazaris, S. Arcidiacono, Y. Huang, et al., *Science* 295 (2002) 472–476.
- [6] H.J. Jin, D.L. Kaplan, *Nature* 424 (2003) 1057–1061.
- [7] A. Seidel, O. Liivak, L.W. Jelinski, *Macromolecules* 31 (1998) 6733–6736.
- [8] K.A. Trabbic, P. Yager, *Macromolecules* 31 (1998) 462–471.
- [9] O. Liivak, A. Blye, N. Shah, L.W. Jelinski, *Macromolecules* 31 (1998) 2947–2951.
- [10] J.M. Yao, H. Masuda, C.H. Zhao, T. Asakura, *Macromolecules* 35 (2002) 6–9.
- [11] I.C. Um, C.S. Ki, H.Y. Kweon, et al., *Int. J. Biol. Macromol.* 34 (2004) 107–119.
- [12] S. Arcidiacono, C.M. Mello, M. Butler, et al., *Macromolecules* 35 (2002) 1262–1266.
- [13] S.W. Ha, Y.H. Park, S.M. Hudson, *Biomacromolecules* 4 (2003) 488–496.
- [14] K. Yamaura, Y. Okumura, A. Ozaki, S. Matsuzawa, *J. Appl. Polym. Sci.: Appl. Polym. Symp.* 41 (1985) 205–220.
- [15] Z.Z. Shao, F. Vollrath, Y. Yang, H.C. Thøgersen, *Macromolecules* 36 (2003) 1157–1161.
- [16] E. Iizuka, *J. Appl. Polym. Sci.: Appl. Polym. Symp.* 41 (1985) 173–185.
- [17] R.S. Lenk, *Polymer Rheology*, Applied Science Publishers, London, England, 1978, p. 77.
- [18] H.G.M. Edwards, D.W. Farwell, *J. Raman Spectrosc.* 26 (1995) 901–909.
- [19] S.D. Zheng, G.X. Li, W.H. Yao, T.Y. Yu, *Appl. Spectrosc.* 43 (1989) 1269–1272.
- [20] P. Monti, G. Freddi, A. Bertoluzza, et al., *J. Raman Spectrosc.* 29 (1998) 297–304.
- [21] S.W. Ha, A.E. Tonelli, S.M. Hudson, *Biomacromolecules* 6 (2005) 1722–1731.
- [22] M. Rossle, P. Panine, V.S. Urban, et al., *Biopolymers* 74 (2004) 316–327.
- [23] X. Chen, D.P. Knight, F. Vollrath, *Biomacromolecules* 3 (2002) 644–648.